Geochimica et Cosmochimica Acta 1964, Vol. 28, pp. 1567 to 1578. Pergamon Press Ltd. Printed in Northern Ireland

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Chemical investigations on soil humic substances

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Abstract—Results of extensive studies on the basic problems of extraction, isolation, fractionation and purification of soil humic substances are presented. Data on the degradation, functional group analysis and molecular weight determination of purified humic fractions from the β-horizon of a podzol are reported, and the role of humic substances in the podzolization process is discussed.

INTRODUCTION

THIS paper is a short summary of investigations initiated by the late Prof. Dr. HANS DEUEL at the Laboratory of Agricultural Chemistry, Swiss Federal Institute of Technology, Zurich, Switzerland.

The chemist working with soil humic substances still awaits the day when he can compare the results of his efforts with those of others who work with well defined, well purified and carefully labeled humic materials isolated by the same techniques. Inasmuch as this is not the case, investigators in this field of soil chemistry are still wandering around in a dense, poorly illuminated jungle of individual incoherent observations. To date these observations have merely shown that soil humic substances are a series of closely related, colored, acidic, probably mainly aromatic substances of variable molecular weight, whose acidity results chiefly from carboxyl groups (DUBACH and MEHTA, 1963). The objective of the chemist working with soil humic substances may be stated as follows:

(1) Elucidation of the general structural features of well purified humic fractions from different soils and horizons by functional group analysis and degradation studies (building blocks and mode of linkage), and establishment of statistical relationships or differences between the various fractions;

(2) Determination of the total amounts of humic substances and their molecular weight distribution to permit the evaluation of the role of humic substances as a factor in soil fertility and in pedological processes. Whereas rather uniform general structural features of diverse humic fractions are possible, their molecular weight distribution has repeatedly been shown to vary considerably and specifically in the different soils. The methods available for such determinations are not yet satisfactory;

(3) Elucidation of the mode of formation of humic substances and of the processes in their decomposition.

It should be clear that real progress in so complicated a field will only be made when many of the rather few investigators will be organized to work on the same problems with exactly the same materials, preferably isolated and distributed by a single laboratory.

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EXTRACTION

With the above aim in mind, considerable time and effort was devoted to the important and basic problems related to extraction and isolation (DUBACH, MEHTA and DEUEL, 1961, 1963).

Alkaline hydroxides have been used most often because of their high extracting power and universal applicability. However, as humic substances are easily altered (oxidation, reduction in light absorption and molecular weight), and non-humic substances, which are later difficult to remove, are co-extracted, alkaline extractants should be avoided. The extraction of lignins, polysaccharides and tannins from plant and organism residues may be avoided to a great extent by a prior elutriation-sieving of the soil sample.

Table 1. Extraction of different soils with acid and with Na_2SO_4 (pH 7 and 8·5), NaOH (0·1 and 0·5 N) and EDTA (pH 7) with and without pre-extraction with acid

Extractant	Hours	Extracted organic carbon in % of total organic carbon			
		Podzol B	Humuskarbonat	Braunerde	Rendzina
6 N HCl	96	—	—	18	—
1 N HCl/1 N HF (1:1)	6	—	22	17	—
1 N HCl/1 N HF (1:1)	48	75	24	22	30
1 N HCl	48	67	16	10	24
HCl/HF;	48	86	32	36	36
0·1 M Na_2SO_4, pH 7	48	—	—	—	—
HCl/HF;	48	92	37	40	41
0·1 M Na_2SO_4; pH 8·5	48	—	—	—	—
HCl/HF;	48	95	53	61	46
0·1 N NaOH	48	—	—	—	—
HCl/HF;	48 × 3	100	90	73	63
0·1 N NaOH	48 × 3	—	—	—	—
4 % EDTA, pH 7	48	92	50	65	37
0·1 M Na_2SO_4; pH 7	48	2	3	3	4
0·1 N NaOH	48	80	30	26	21

Milder and more selective extraction procedures using neutral or slightly alkaline solvents have been extensively studied. Alkali salts of complexing agents, such as pyrophosphoric acid and EDTA, are most suitable, however, they are often much less effective than alkali hydroxides. The effectiveness of the extractants can be increased by a thorough pretreatment of the soil with acids (HCl/HF) to destroy carbonates and silicates.

Inasmuch as the alkali salts of humic substances, with the exception of the highest molecular weight fractions, are soluble at pH 7–8, a major part of the humic substances should be extractable at this pH provided the polyvalent cations have been removed, e.g. by prior acid extraction of the soil.

Table 1 shows the results of experiments related to this problem. Prior to extraction the soils were passed through an 0·06-mm sieve by a special elutriation sieve technique. Soils differing widely in pH and clay content were selected to test the universal applicability of the extraction procedures. As had earlier been reported by others, a substantial part of the organic carbon was extracted with acids. Mixtures of HCl and HF were more effective than HCl alone; 1N HCl/1N HF

extracted the same amount of organic carbon in 6 hr as did 6N HCl in 96 hr. This fact excludes the possibility of extraction by degradation. We could detect no alteration of humic substances in 1N HCl or HF at room temperature: neither the light absorption nor the molecular weight distribution of fulvic acids in 1N HCl changed during one month of observation, as shall be discussed below.

After acid extraction, further portions of organic carbon were extracted by neutralization of the soil residue in 0·1M Na_2SO_4 with NaOH to pH 7 and 8·5, and additional amounts by extraction with 0·1N NaOH. A thrice repeated extraction process with 1N HCl/1N HF, and with 0·5 NaOH, still left considerable organic carbon at the residues.

Four per cent EDTA at pH 7 was as effective an extractant as 0·1N NaOH with acid pretreatment, and considerably better than 0·1N NaOH without acid pretreatment. Practically no organic carbon was extracted by Na_2SO_4 without acid pretreatment.

The extracted humic substances have to be isolated from acid and from neutral or slightly alkaline extracts. Inasmuch as the humic acids can easily be isolated from all extracts by precipitation and dialysis, the problem is reduced to the isolation of fulvic acids; the large amount of extractant has to be separated from the relatively small amount of fulvic acid present in the acid extracts and the acidified solutions of the neutral or alkaline extracts. The ionic extractants may be removed by desalting with exchangers, however, only at the cost of a substantial loss of humic material. It is preferable to remove the fulvic acids from the acidified extracts by adsorption on carbon or on cellulose anion exchangers; their desorption, however, requires an alkaline eluant. The fulvic acids may also be isolated by precipitation as insoluble Ba, Cu or Fe salts, provided that the extractant is not co-precipitated (insoluble salts with pyrophosphoric acid and HF). This last technique, consisting of the precipitation of the fulvic acid at pH 2–3 with the polyvalent cations present in the extract or by added $FeCl_3$, followed by thorough dialysis and decomposition of the fulvic acid salts by shaking with strongly acidic cation exchangers, has been found to be the easier method for isolating even traces of fulvic acids from HCl and EDTA extracts.

Extraction with 1N HCl followed by extraction at pH 7 and in progressively more alkaline media with sodium salt buffers can be recommended as a universally applicable technique that permits the easy isolation of humic material of the whole molecular weight range. Extraction with EDTA at pH 7 and successively higher pH values is the preferable method when any acid treatment of the humic substances must be strictly avoided for special requirements of the investigations intended.

Purification

It has been postulated that carbohydrates and nitrogen compounds are an integral part of the humic molecule (Mehta, Dubach and Deuel, 1961). This question has been investigated by attempting to remove these two classes of compounds from fulvic acids by mild physical methods (Roulet *et al.*, 1963).

Fulvic acid preparations from Braunerde (FS-BE), humus carbonate soils (FS-HKB) and Rendzina (FS-RE), in which the carbohydrate content of about 25 per cent could not be reduced by repeated precipitation, were fractionated by gel-filtration on Sephadex (Fig. 1). The uronic acid content, the nitrogen content,

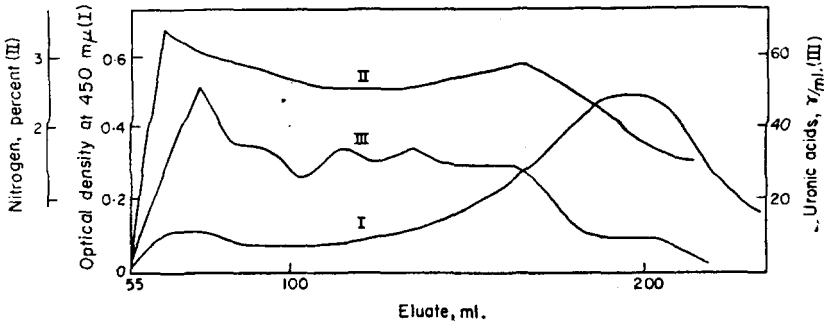

Fig. 1. Fractionation of fulvic acid FS-HKB on Sephadex G-75 with 0·2 M acetic acid.

Table 2. Uronic acid content and optical density of fulvic acids before and after fractionation by Sephadex G-75 gel filtration

Material	Weight (mg)	Uronic acids (%)	Optical density at 450 mμ ($E^{1\%}_{1cm}$)
Humuskarbonatboden			
FS-HKB	1000	10	6·6
fraction 1	158	18	3·1
fraction 2	367	12·5	4·4
fraction 3	405	2·0	9·0
Rendzina			
FS-RE	1000	10	6·7
fraction 1	320	20	2·4
fraction 2	395	8	7·2
fraction 3	230	1·7	13·1
Braunerde			
FS-BE	1000	10	4·2
fraction 1	150	21	1·2
fraction 2	415	14	2·8
fraction 3	267	1·0	6·2

and absorption at 450 mμ were measured in the eluate. As can be seen, the absorption curve, which represents the humic substances, has a different shape from that of the uronic acid curve, and the maxima of both do not coincide. This means that at least the major part of the carbohydrates is not chemically linked with humic substances. No such clear cut result was obtained with regard to the nitrogen compounds, although decrease in the nitrogen content of the fractions with increasing content of humic material points in the same direction. No change in the amino acid composition was observed in the various fractions.

Three major fractions were taken: (1) from around the maximum of the uronic acid curve; (2) from around the maximum of the humic substance curve, and (3) from the tubes in between. Table 2 shows the uronic acid content and the absorption of these fractions. As uronic acid times 2·5 gives a good value for the total carbohydrate content in humic materials, it is apparent that carbohydrates were reduced from 25 to under 5 per cent by this preparative fractionation. As a result of purification, the absorption of fraction 3 was considerably increased.

The Sephadex gel which was used is a cross-linked dextran that permits molecules up to a certain size access to the interior of the gel grains. Sephadex preparations G-25, G-50, G-75 and G-100 exclude molecules of molecular weights greater than 3500–4500, 8000–10,000, 40,000–50,000 and 100,000 respectively. Molecules with no access to the interior of the gel grains are eluted from the outer or void volume of the Sephadex column (V_0). The elution of smaller molecules entering the gel

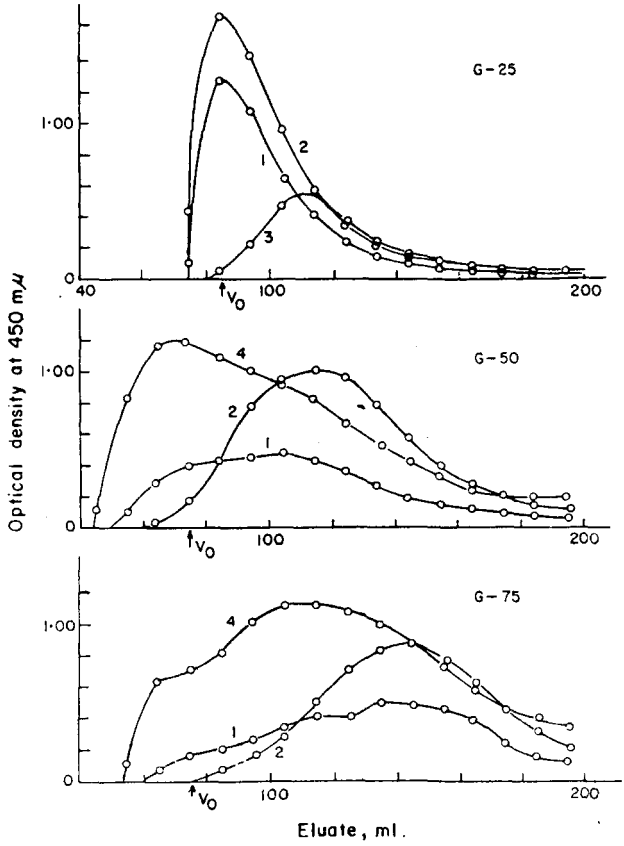

Fig. 2. Elution curves of the fulvic acids FS-1 (1), FS-2 (2) and FS-3 (3) and of the humic acid B-HS (4) in Sephadex G-25, G-50 and G-75. V_0 is void volume.

grains is increasingly retarded with decreasing molecular weight. If, thus, estimation of the molecular weights is attempted, any adsorption of the material on the gel grains will cause the estimate to be too low. Whereas no adsorption was observed with fulvic acid preparations in glycine–NaOH buffer, small adsorption was encountered in experiments with brown humic acids and, unfortunately, extensive adsorption with gray humic acids.

The fulvic acid fraction, FS-2, soluble in tetrahydrofuran (THF), could not be fractionated into clear cut fractions on Sephadex gels G-25, G-50 and G-75 (Fig. 2). The elution curves always had only one maximum, which was, in the order mentioned, progressively displaced to the right, demonstrating increased penetration

into the interior of the gel grains. If tailing is neglected, FS-2 is nearly completely excluded from G-25 (maximum at V_0, asymmetric curve), but enters into the gel grains of G-50. Thus, about 90 per cent of this material has a molecular weight range of 4000 to 9000; the average molecular weight may be estimated to be about 5000.

No definite fractions, as well, were obtained for the other fulvic acid fractions (FS-1, insoluble in THF, and FS-3, soluble in a THF/ether mixture). As FS-1 is excluded completely from G-25, partially from G-50, and enters G-75 completely, its molecular weight range is about 4000 to 40,000, the average molecular weight may be about 6000. FS-3 completely entered into G-25 and may thus have a molecular weight range of 2000 to 4000.

The brown humic acid, B-HS, enters G-50 only partially, the main part of it enters G-75, and a small amount is excluded from G-100. The molecular weight range is, therefore, from 5000 to over 100,000 (MEHTA, DUBACH and DEUEL, 1963b).

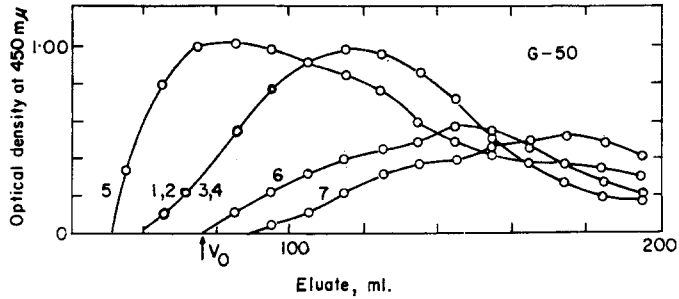

Fig. 3. Elution curves of the fulvic acid FS-Ba on Sephadex G-50 before and after various treatments (1) untreated; (2 and 3) after drying under high vacuum at 40 and 100°C. for 60 hr; (4) after 1 month in 1 N HCl at room temperature; (5) after 16 hr boiling in 6 N HCl; (6) after 1 month in 1 N NaOH at room temperature; (7) after 3 hr in 6 N NaOH at 250°C.

Although adsorption tends to give low estimates, the Sephadex technique provides valuable information on the molecular weight range and allows a rather good and easy estimate to be made of the average molecular weight.

The Sephadex technique is also suited to recognition of any changes in the molecular weight distribution of humic substances that may occur on various treatments (MEHTA, DUBACH and DEUEL, 1963b). Figure 3 shows that the molecular weight distribution of a fulvic acid preparation isolated at pH 7 with EDTA was not changed either after drying for 60 hr at 40 and 100°C, nor after treatment, as mentioned earlier, with 1N HCl at room temperature for one month. Boiling with 6N HCl caused a considerable increase in molecular weight, whereas in alkali at 250°C the average molecular weight decreased. Sephadex gel filtration may be particularly helpful if the analytical results on various humic fractions are to be compared. Such comparisons are only justified if the preparations have the same or similar molecular weight distribution patterns. Such patterns should be used as an additional label for humic, and especially fulvic acid preparations.

The possibility of removing at least a substantial part of the carbohydrates and

proteins from humic substances, has, therefore, been demonstrated. However, better preparative methods should still be sought.

The greatest problems in the purification process begin with aromatic compounds such as lignins, degraded lignins, tannins and microbial metabolites where the line of separation from humic substances is impossible to define on paper or to execute in practice. Here the process of purification merges imperceptibly with that of fractionation. If such closely related impurities are present in humic substances, they should be enriched in some fractions and eliminated in others. In actual fact, in spite of the most intensive efforts, no discrete fractions have ever been isolated from reasonably purified humic preparations. The methods used to characterize the fractions (elementary analysis, equivalent weight, i.r.- and u.v.-spectra) show rather a continuous variation. These are, however, poor criteria, and it is possible that with the more sensitive methods of functional group analysis and degradation more significant differences between the fractions will be found and the fractionation–purification procedures will be refined further.

Table 3. Estimate of yield of products obtained in the degradation of humic substances (fulvic and humic acids from podzol B) and lignin with 5 N NaOH at 170° for 3 hr

	Humic substances		Lignin	
Degradation products	170°C (‰)	250°C (‰)	170°C (‰)	250°C (‰)
catechol	0·5	7·5	—	17·5
resorcinol	0·5	5·0	—	—
4-hydroxybenzoic acid	1	—	0·5	—
3-hydroxybenzoic acid	1	7·5	—	—
4-hydroxybenzaldehyde	0·5	—	—	—
3,4-dihydroxybenzoic acid	1	—	—	—
3,5-dihydroxybenzoic acid	3	7·5	—	—
vanillic acid	2	—	6	—
vanillin	1	—	6	—
ferulic acid	—	—	2	—
syringic acid	—	—	1	—
syringaldehyde	—	—	2	—

Degradation

Thus far we have been unsuccessful in obtaining different degradation products from various humic fractions of the podzol B horizon (Jakab *et al.*, 1962, 1963; Mehta *et al.*, 1963a). Table 3 shows the phenolic degradation products and an estimate of their yield. The compounds were obtained from various fulvic and humic acid fractions from the podzol B horizon, and from a spruce lignin preparation treated with 5N NaOH at 170 and 250°C (Jakab *et al.*, 1963). The following products were identified: catechol, resorcinol, vanillin, 4-hydroxybenzaldehyde, 3- and 4-hydroxybenzoic acid, 3,4- and 3,5-dihydroxybenzoic acids and vanillic acid. The possibility of artifact formation in alkaline medium seems excluded since other workers recently reported all the nitrated analogues by degradation with HNO_3. Thus, the considerable yields of mono- and diphenolic degradation products strongly suggest a primarily ether-linked aromatic system. Most informative are the identifications of compounds such as *m*-hydroxybenzoic acid, 3,5-dihydroxybenzoic acid

and resorcinol, which are not obtained from lignin by the same degradation method and which are incompatible with the present concepts of the structure of lignin.

Contrary to other reports, no C_6–C_3 compounds were found among the degradation products of our podzol B humic preparations, even when many other degradation procedures were used (JAKAB *et al.*, 1962, 1963; MEHTA *et al.*, 1963a).

FUNCTIONAL GROUPS

Carboxyl groups, hydroxyquinones, phenols and enols have been thought responsible for the acidity of humic substances. As the acidity of these groups overlaps (2,5-dihydroxyquinone is more acidic than acetic acid), they cannot be distinguished

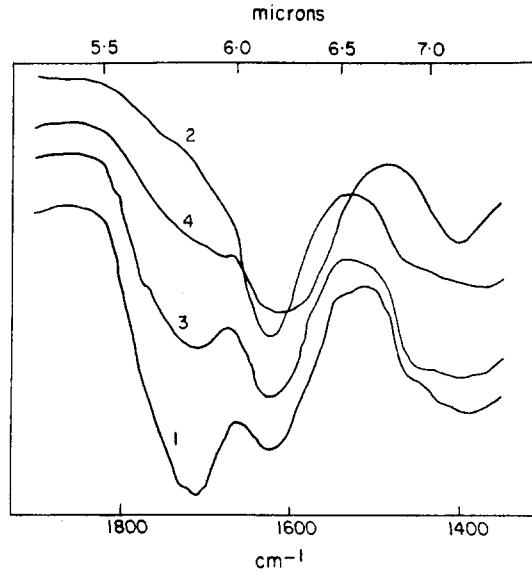

Fig. 4. Infrared spectra of fulvic acid FS-2; (1) FS-2, (2) FS-2-Na-salt; (3) FS-2 after 1 week reduction with B_2H_6; (4) after 1 month reduction.

by pK-dependent methods such as titrations in aqueous and non-aqueous media (which have been often used), reactions with calcium acetate, iodide–iodate and barium hydroxides, and by methylation or saponification. In addition, there is a variation of the pK-values within the groups because of the polybasicity of the material. The presence of carboxyl groups (Fig. 4) has been established by infrared spectroscopy (shift of the major part of the carbonyl adsorption band from 5·7 to 5·9, to 6·1 to 6·3 μ).

The following description demonstrates attempts to determine carboxyl groups by a strictly non-pK-dependent method (MARTIN, DUBACH, MEHTA and DEUEL, 1963). The carboxyl groups of the tetrahydrofuran-soluble fulvic acid fraction (FS-2) were reduced with diborane, and changes in the elementary composition and in acetylatable OH-groups were determined. The reduction was followed by infrared spectroscopy. Figure 4 shows the decrease in absorption in the carbonyl region after one week and after one month of reduction with B_2H_6. As can be seen, the fulvic acid still displayed diffuse absorption in the carbonyl region after reduction

Table 4. Analytical data for fulvic acid (FS-2) before and after reduction (1 month) with diborane (FS-2-R)

Material	C		H		O		N	OCH_3	OH	>C=O
	(%)	(m-equiv./g)	(%)	(m-equiv./g)	(%)	(m-equiv./g)	(%)	(m-equiv./g)	(m-equiv./g)	(m-equiv./g)
FS-2	51	42	3·6	36	44	27·5	0·8	0·04	3·6	1
FS-2-R	57	47·6	5·7	57	35·8	22·4	1·2	0·02	10·5	0
FS-2-R*	(51)	(42)	5·1	51	32·0	20·0	—	—	9·4	;0

FS-2-R*: calculated for C-content of FS-2

e.g. oxygen $= \frac{22{\cdot}4}{57} \cdot 51 = 20$ m-equiv./g.

for one month. This absorption, however, remained unchanged after formation of the Na-salt, demonstrating the complete reduction of the carboxyl groups.

In Table 4 the analytical data for the starting material and the reduced fulvic acid are compared. Since after reduction only the number of carbon atoms remains unchanged, corrected data on the same carbon basis must be compared (FS-2-R*). The hydrogen content increased on reduction by 15 m-equiv./g, and the oxygen content decreased by 7·5 m-equiv./g. This would correspond to a carboxyl content of 7·5 m-equiv./g. No carbonyl groups were found in the reduced preparation in spite of the remaining diffuse infrared absorption in the carbonyl region. After correction for reduced carbonyl groups, the carboxyl content based on the hydrogen increase is 6·5 m-equiv./g, by the oxygen decrease it is 7·5 m-equiv./g, and by the increase in acetylatable OH-groups it is 4·8 m-equiv./g. Because of possibly incomplete acetylation, 4·8 m-equiv. should be regarded as a minimum value. The possible elimination of non-carboxyl oxygen by reduction with diborane makes 7·5 m-equiv. a maximum value. Titration with NaOH to pH 8·5 gives 5·8 m-equiv., and reaction with barium acetate gave 7·8 m-equiv./g of acidic groups. Aside from the carboxyl groups determined by diborane, there is scarcely any room for strongly acidic hydroxyquinones and enols.

The functional groups were also determined on a series of fulvic acids which were obtained by fractional dialysis of an HCl-extract from the podzol B horizon. Table 5 shows the light absorption and its considerable increase with time of dialysis from fraction D_1 to fraction D_{13}. For comparison, the light absorption of FS-2 and of the brown humic acid fraction B-HS are also given; absorption of FS-2 is about that of D_{10}. Figure 5 demonstrates the gel-filtration patterns of these dialysis fractions on Sephadex G-50. With increasing light absorption in the fractions D_1 to D_{13} there is a parallel increase in the average molecular weight, demonstrated by the shift of the maxima of the elution curves from the right to the left. The analytical data for these fractions are given in Table 6.

One mol of hydrogen is evolved with diborane per mole of carboxyl or hydroxyl groups. This active hydrogen, as determined by diborane, decreases with increasing average molecular weight of the fractions and agrees well with the total acidity measured by reaction with $Ba(OH)_2$. This observation excludes the presence of alcoholic hydroxyl groups. If this is so, it is possible to calculate the carboxyl groups by subtracting the OH-groups determined by acetylation from the active hydrogen. The difference between this value and the carboxyl value obtained by the pK-dependent reaction with barium acetate increases as the content of hydroxyl

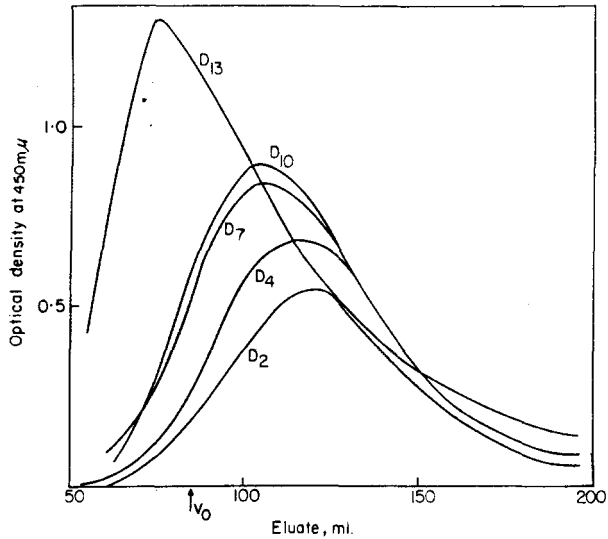

Fig. 5. Molecular weight distribution of fulvic acid fractions D_2–D_{13} on Sephadex G.-50.

Table 5. Fractional dialysis of fulvic acid FS-HCl (20 g)

Fraction	Time of dialysis (hr)	Yield (g)	Optical density at 450 mμ ($E^{1\%}_{1cm}$)
D_1	1	0·772	9·5
D_2	16	3·709	10·0
D_3	19	0·623	10·3
D_4	65	5·220	11·7
D_5	73	0·608	13·2
D_6	88	0·800	13·8
D_7	121	1·137	16·6
D_8	137	0·761	18·3
D_9	184	0·637	18·4
D_{10}	328	1·009	19·9
D_{11}	383	0·924	23·0
D_{12}	472	0·556	25·4
D_{13}	residue	2·202	28·5
FS-2	—	—	20·0
B-HS	—	—	64·0

Table 6. Analytical data for fulvic acid fractions D_2, D_4, D_7, (FS-2), D_{10}, D_{13}, and for brown humic acid B-HS, in m-equiv./g

Material	Active H (diborane) (1)	Total acidity ($Ba(OH)_2$) (2)	Hydroxyl (acetylation) (3)	Carboxyl $Ba(OAc)_2$ (4)	Carboxyl (1)-(3) (5)	Carbonyl (6)
D_2	13·0	12·0	2·0	10·3	11·0	2·5
D_4	12·4	11·6	3·4	9·9	9·0	2·5
D_7	10·6	10·2	3·5	8·6	7·1	2·4
FS-2	11·6	11·7	3·6	7·8	8·0	1·0
D_{10}	9·6	9·9	4·4	8·0	5·2	2·4
D_{13}	9·0	8·8	5·6	5·9	3·4	2·4
B-HS	7·1	8·6	4·3	5·3	2·8	1·9

groups in the fractions increases. This points to the possibility of barium acetate reacting with hydroxyl groups leading to excessively high carboxyl values. The carbonyl contents of the fractions remained at a low and rather constant value.

An interpretation of these results is shown in Table 7. The assumption was tested as to whether these fractions, displaying a great variation in the content of functional groups, have, nevertheless, after elimination of the functional groups, a skeleton of the same composition. This assumption would be confirmed if the C/O ratio in such a skeleton showed a constant value. As can be seen, this value varies from 3 to 8·5. However, we still prefer to blame the analytical methods, rather than to reject this stimulating assumption.

Table 7. Interpretation of results of functional group analysis of fulvic acid fractions D_2, D_4, FS-2, D_{10}, D_{13} and of brown humic acid B-HS (m-equiv./g)

Material	C	O	"Skeleton"-C	"Skeleton"-O	C/O in "Skeleton"
D_2	40·0	30·0	29·0	3·5	8·5
D_4	40·6	30·0	31·6	6·1	5·2
D_7	42·0	28·2	34·9	8·1	4·3
FS-2	42·0	27·5	34·0	6·9	4·9
D_{10}	41·7	28·1	36·5	10·9	3·7
D_{13}	42·0	28·2	38·6	13·4	2·9
B-HS	47·5	23·2	44·7	11·4	3·9

Benzene nuclei connected by 2 ether linkages: C/O = 6.
Benzene nuclei connected by 4 ether linkages: C/O = 3.

The residual or skeletal oxygen not accounted for in the functional groups increases with molecular weight. In fraction D_{13}, for example, 50 per cent of the oxygen was not accounted for in the functional group analysis. The degradation studies suggest an aromatic-benzenoid skeleton. If, for example, benzene is taken as the main building block, the C/O ratio of 3 to 8·5 found in the skeleton would require up to 4 ether linkages per aromatic ring. This seems to be an impossibly high number. In this connection it may be remembered that after reduction with diborane a residual absorption in the carbonyl region of the infrared spectrum was still observed. However, no carbonyl groups could be determined in the materials reduced by $NaBH_4$. Is this infrared absorption caused by extremely stable lactones or quinones?

Podzol Formation

To conclude, let us discuss a more practical aspect of humic substance research. While extracting the materials from the podzol soils, we often pondered on the podzolization process leading to the formation of those astonishingly clear cut horizons. In this respect, we think that one has to consider that humic substances are only precipitated from solution by polyvalent cations after reaching a certain point of saturation, this point being reached later in fractions of smaller average molecular weight. If conditions, such as a parent material poor in bases and a climate high in rainfall prevail, the humic substances constantly produced by the decaying plant material travel down the profile a certain distance until they become saturated with polyvalent cations and are precipitated to form a distinct zone of accumulation.

The distance traveled is short at the beginning of the podzolization process. As the process continues and the upper part of the profile is depleted of available polyvalent cations, the zone of accumulation progressively moves downwards. This is probably caused by the fact that the humic substances constantly arriving in the accumulation zone are now less and less saturated with polyvalent cations and are thus able to mobilize the already precipitated humic substances by removing part of the precipitating ions. In fact, isolated fulvic acids were found to be effective in extracting humic substances from podzol B soil samples. It seems possible to explain readily in this way all the special features of the podzolization process.

Acknowledgements—We wish to thank the *Schweizerischer Nationalfonds zur Förderung wissenschaftlicher Forschung*, the *Fonds der Wald und Holzforschung* and the *Consejo Superior de Investigaciones Cientificas de Espana* for financial support.

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